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GC 2400 Sample Analysis Report

Analysis of higher alcohols in wine, port wine and brandy by FET HS-GC-FID

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Content

Analytical method for the analysis of higher alcohols

Short description

For the analysis of higher alcohols in alcoholic beverages, several analytical methods can be found in literature and standardized methods. Typically gas chromatography is used with either a direct liquid injection or a headspace injection for the sample introduction. In this report a headspace (HS) GC-FID method is described. More specifically, the total vaporization technique (TVT) or full evaporation technique (FET) is used for the headspace sample preparation. In this case, only a small sample volume is added to the headspace vial, typically less than 20 µL. When the vial is heated during thermostatting inside the HS oven, the sample evaporates completely, leaving only gas phase inside the HS vial. As a consequence, there is no equilibrium between the sample phase and the gas phase, and one single calibration method can be used for different sample types.

Method settings for TVT-GC-FID

GC METHOD	
Parameter	**Setting**
Injector temperature	150°C
Injector pressure	21 psi
Split flow	5 mL/min
Column	Elite-WAX ETR, 60 m x 0.32 mmID x 1.0 µm df
GC oven program	40°C (hold 0.5 min), 5°C/min to 150°C, 15°C/min to 180°C (hold 0.5 min)
GC run time	25 min
FID temperature	250°C
FID air flow	400 mL/min
FID H_2 flow	30 mL/min
FID make-up gas (N_2) flow	25 mL/min

HS METHOD	
Parameter	**Setting**
Oven temperature	120°C
Needle temperature	125°C
Transfer line temperature	135°C
HS pressure	25 psi
Thermostatting time	15.0 min
Pressurization time	2.0 min
Injection time	0.04 min
Withdraw time	0.5 min
Vial size	20 mL
Sample volume	10 µL

Method calibration

Calibration standard and analytes

For the calibration of the method a stock standard solution, available at the time of analysis, is used to prepare a set of calibration standards. The table below shows the composition of this stock standard solution.

	STOCK STANDARD SOLUTION – HIGHER ALCOHOLS IN ETOH/H2O	
	Analyte	**Concentration (ppm)**
1	acetaldehyde	780
2	ethyl acetate	900
3	methanol	791
4	n-propanol	800
5	2-methyl-1-butanol	815
6	3-methyl-1-butanol	830
7	n-pentanol	816
9	n-hexanol	820
	Solvent	$EtOH/H_2O$ (50:50)

Preparation of calibration standards

A series of calibration standards are prepared by dilutions from the stock standard solution in pure water (MilliQ). The table below shows the dilutions for the preparation of the calibration standards and its concentrations. The stock standard solution itself is also used in the calibration as the high-level calibration standard.

	vol. Stock Std. Sol. (µL)	**vol. H_2O (µL)**	**Total vol. (µL)**	**dilution factor (x)**
STD5	200	800	1000	5
STD4	100	900	1000	10
STD3	50	950	1000	20
STD2	25	975	1000	40
STD1	20	980	1000	50

	Conc. Stock Std. Sol. (ppm)	Conc. STD 1 (ppm)	Conc. STD 2 (ppm)	Conc. STD 3 (ppm)	Conc. STD 4 (ppm)	Conc. STD 5 (ppm)
acetaldehyde	780	15.6	19.5	39.0	78.0	156.0
ethyl acetate	900	18.0	22.5	45.0	90.0	180.0
methanol	791	15.8	19.8	39.6	79.1	158.2
n-propanol	800	16.0	20.0	40.0	80.0	160.0
2-methyl-1-butanol	815	16.3	20.4	40.8	81.5	163.0
3-methyl-1-butanol	830	16.6	20.8	41.5	83.0	166.0
amyl alcohols*	1645	32.9	41.1	82.3	164.5	329.0
n-pentanol	816	16.3	20.4	40.8	81.6	163.2
n-hexanol	820	16.4	20.5	41.0	82.0	164.0

*2-methyl-1-butanol and 3-methyl-1-butanol coelute on the column and are quantified as their sum

Calibration settings and results

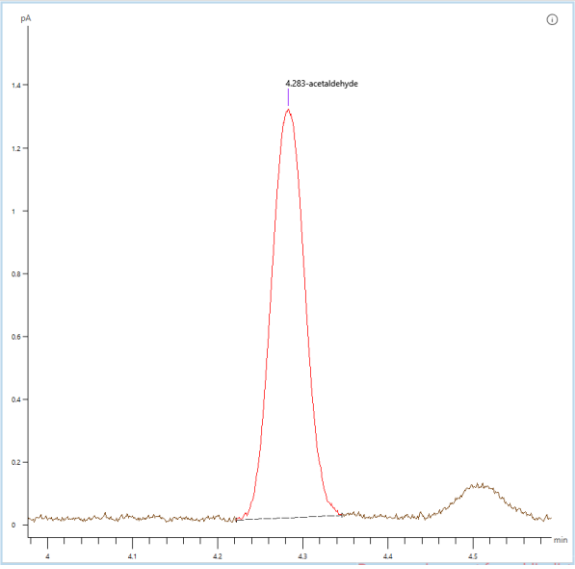
Using all 6 calibration standards, calibration curves for all analytes are calculated in SimplicityChrom software, according to the processing parameters as shown in the table below.

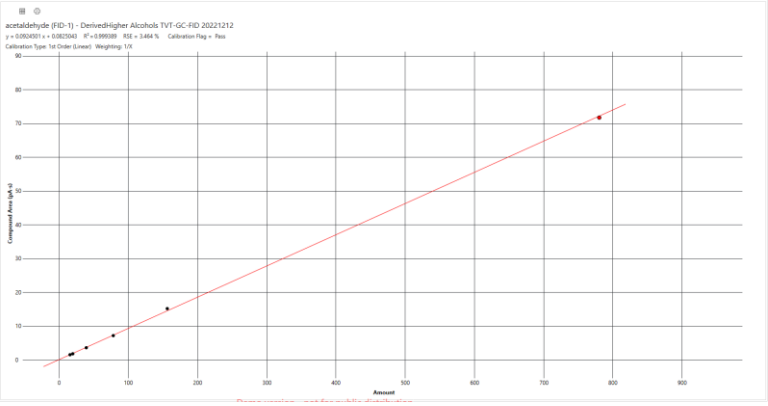
Compounds	Expected RT (min)	Response	Calibration Type	Origin Treatment	Weighting Factor
acetaldehyde	4.273	Area	1st Order (Linear)	None	1/X
ethyl acetate	7.385	Area	1st Order (Linear)	None	1/X
methanol	7.571	Area	1st Order (Linear)	None	1/X
n-propanol	11.468	Area	1st Order (Linear)	None	1/X
amyl alcohols	16.899	Area	1st Order (Linear)	None	None
n-pentanol	18.304	Area	1st Order (Linear)	None	1/X
n-hexanol	21.557	Area	1st Order (Linear)	None	1/X

Example peak integrations and calibration curves for all analytes are shown below.

CALIBRATION RESULT - ACETALDEHYDE

Calibration curve

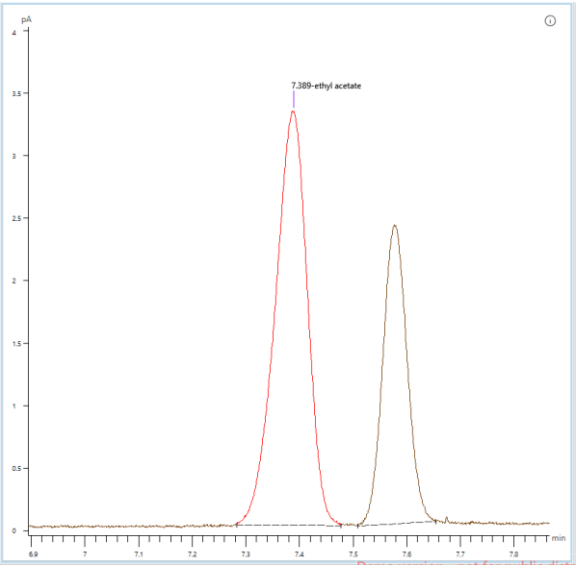


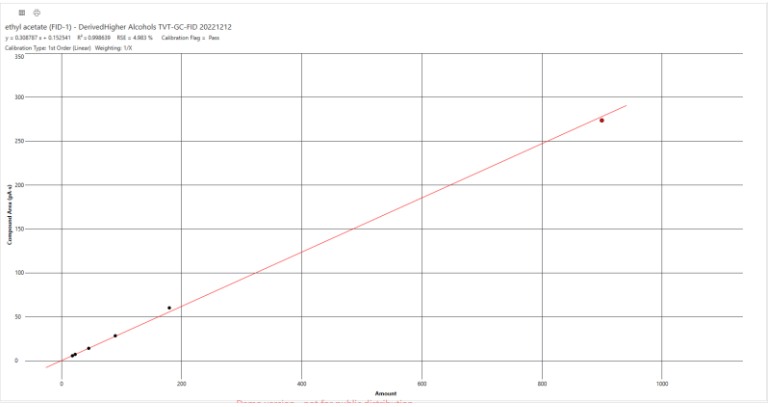


Example peak integration from STD3

CALIBRATION RESULT – ETHYL ACETATE

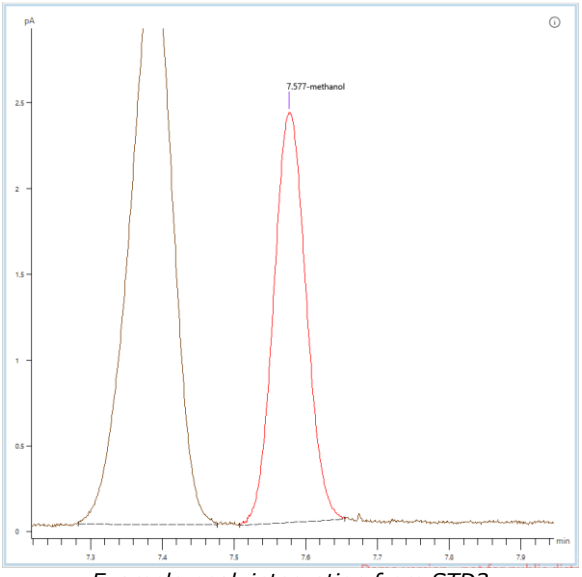
Calibration curve





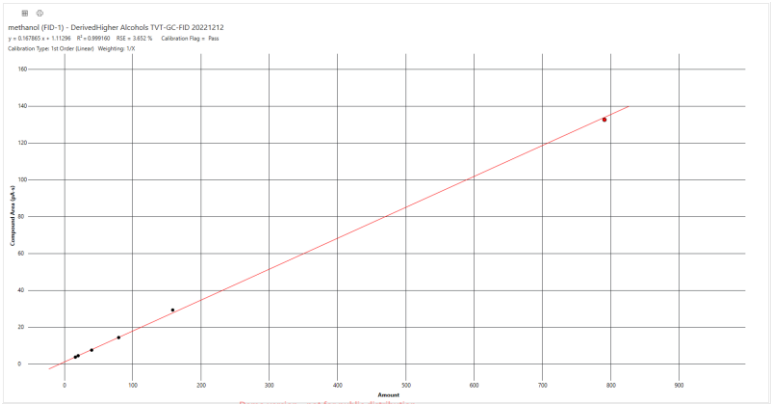
Example peak integration from STD3

CALIBRATION RESULT – METHANOL

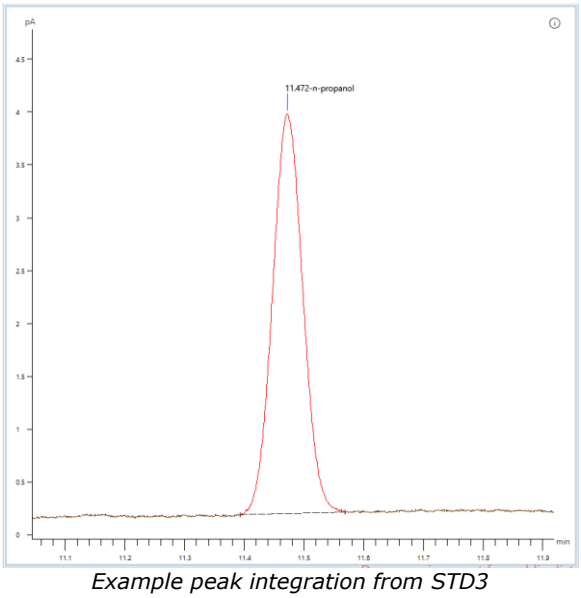


Example peak integration from STD3

Calibration curve

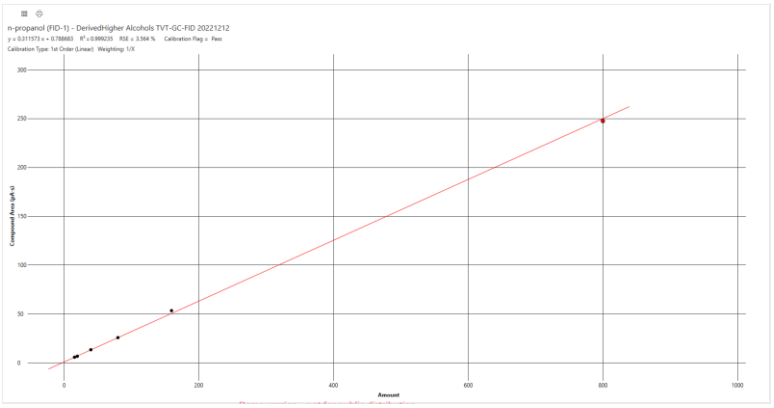


CALIBRATION RESULT – N-PROPANOL



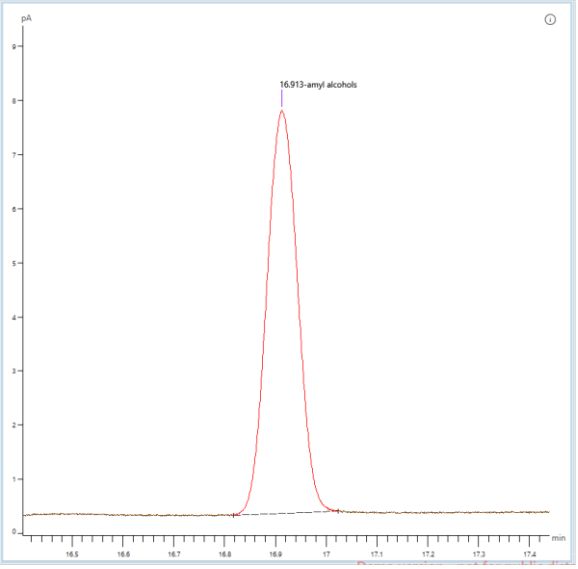
Example peak integration from STD3

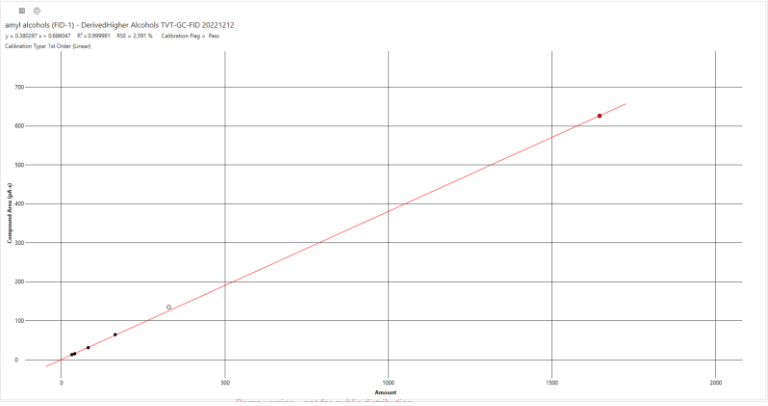
Calibration curve



CALIBRATION RESULT – AMYL ALCOHOLS (2-METHYL-1-BUTANOL + 3-METHYL-1-BUTANOL)

Calibration curve

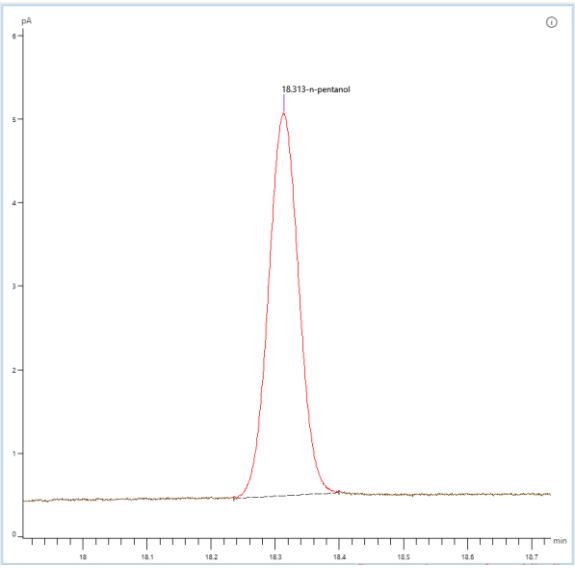


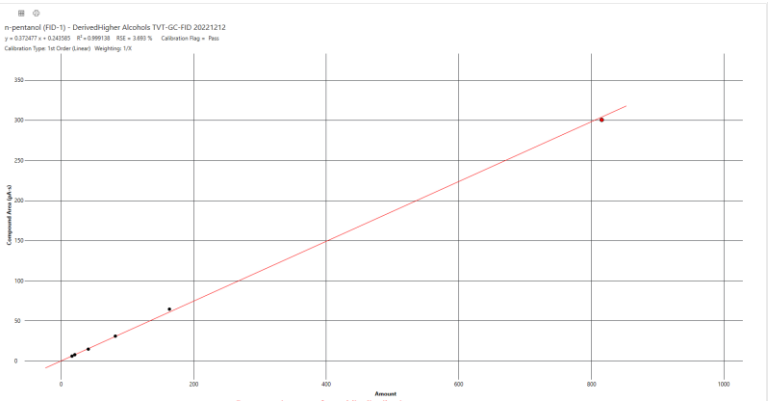


Example peak integration from STD3

CALIBRATION RESULT – N-PENTANOL

Calibration curve

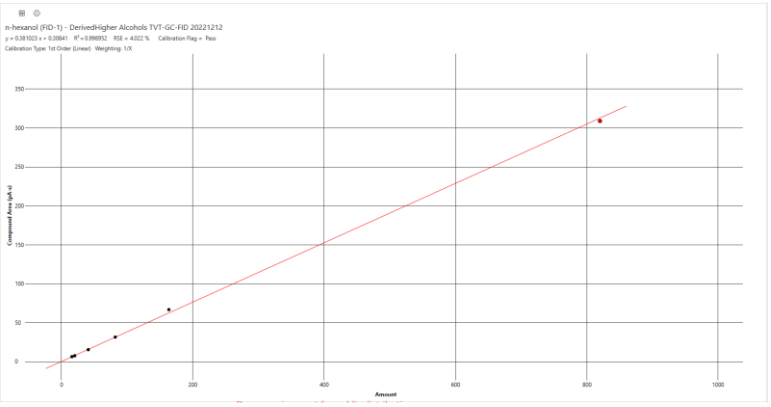


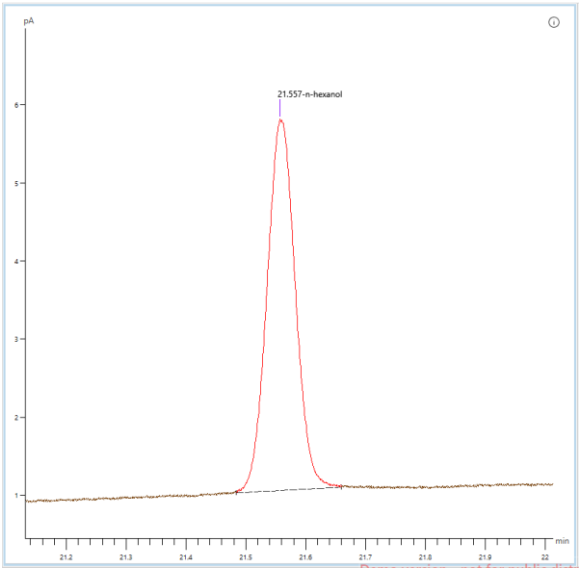


Example peak integration from STD3

CALIBRATION RESULT – N-HEXANOL

Calibration curve

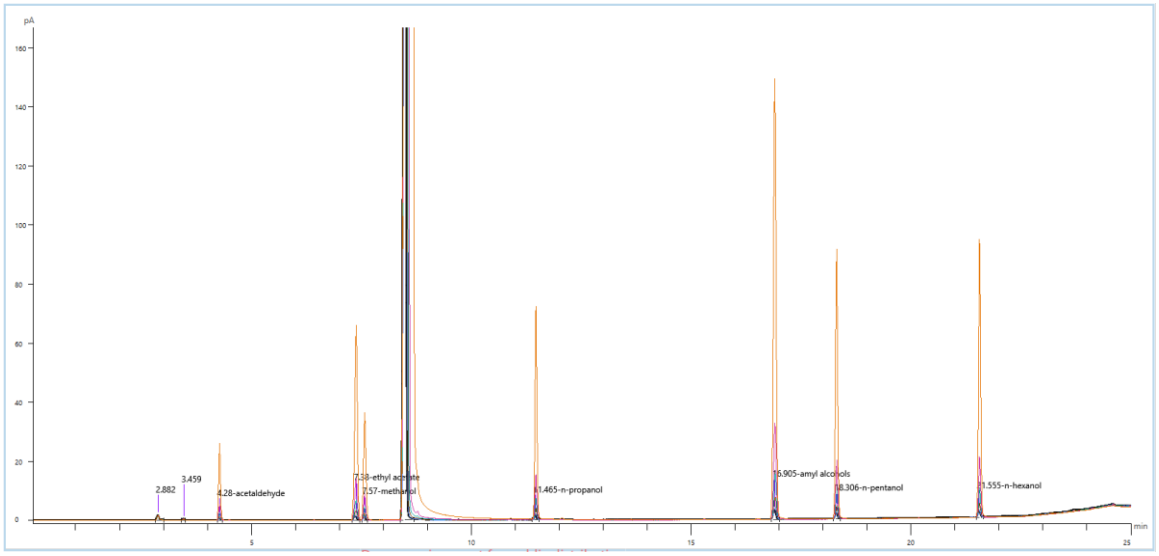




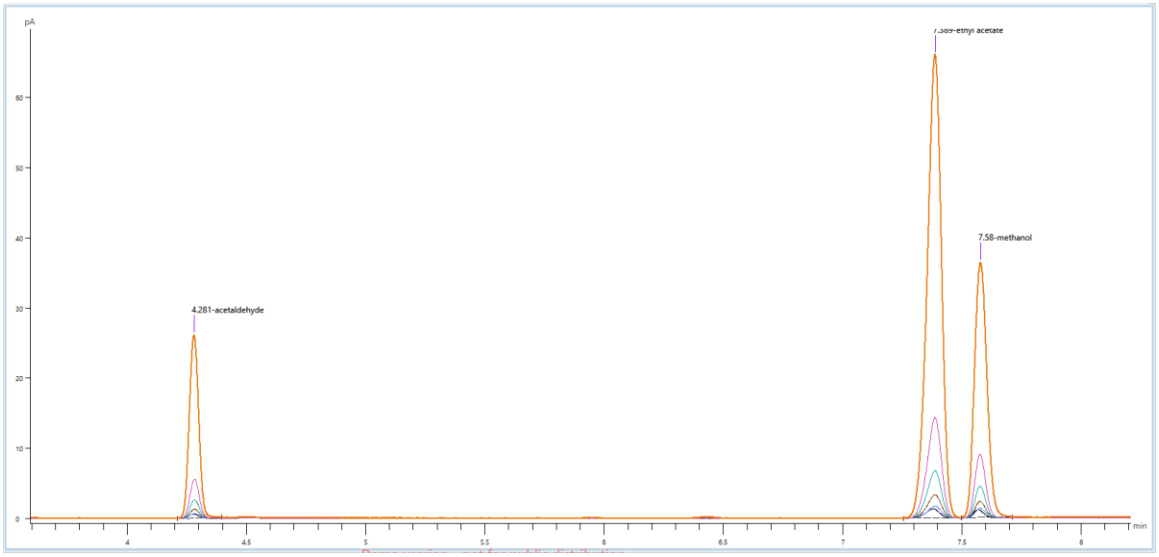
Example peak integration from STD3

Calibration standard chromatograms

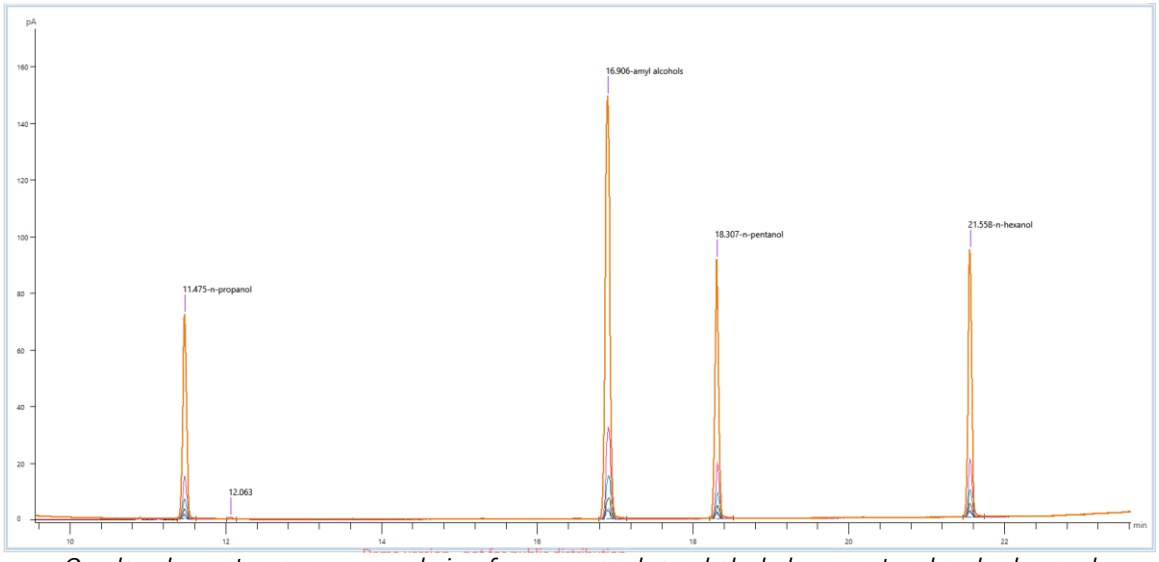
The images below show an overlay of the 6 calibration standard chromatograms and zoomed views of the analytes of interest in front of and after the ethanol peak.



Overlay chromatogram of all 6 calibration standards



Overlay chromatogram – zoomed view for acetaldehyde, ethyl acetate and methanol



Overlay chromatogram – zoomed view for n-propanol, amyl alcohols, n-pentanol and n-hexanol

Calibration summary

Peak Name	Retention Time (min)	R^2	RSE (%)	Calibration Flag
acetaldehyde	4.2797967	0.99939	3.46424	Pass
ethyl acetate	7.3802722	0.99864	4.98279	Pass
methanol	7.5701795	0.99916	3.65212	Pass
n-propanol	11.464792	0.99924	3.56421	Pass
amyl alcohols	16.905256	0.99999	2.59124	Pass
n-pentanol	18.306389	0.99914	3.69317	Pass
n-hexanol	21.554701	0.99895	4.0217	Pass

Sample analysis results

Sample description

A total of 8 samples are presented for analysis, including wine, port wine and brandy. A short description of each sample can be found in the table below.

Sample#	Product	Alcohol (%vol at 20ºC)
41648	Red Wine	13,80
41649	Red Wine	13,86
41257	Red Port Wine	19,74
41664	Red Port Wine	19,98
41255	White Port Wine	19,31
41831	Brandy	36,37
41832	Brandy	36,26
41276	Brandy	37,87

8 alcoholic beverages for sample analysis of higher alcohols by TVT-GC-FID

Results per sample

The table below shows the sample analysis results presented for each sample individually.

SAMPLE RESULT - Red Wine 41648

Peak Name	Retention Time (min)	Area (pA·s)	Conc. (ppm)	Calibration In Range	R^2	RSE (%)	Calibration Flag
acetaldehyde	4.28295	7.4352275	79.531767	In Range	0.99939	3.46424	Pass
ethyl acetate	7.392872	17.715774	56.878091	In Range	0.99864	4.98279	Pass
methanol	7.5759954	30.979996	177.92265	In Range	0.99916	3.65212	Pass
n-propanol	11.470724	9.7547181	28.776651	In Range	0.99924	3.56421	Pass
amyl alcohols	16.919089	102.11756	266.71643	In Range	0.99999	2.59124	Pass
n-hexanol	21.598748	26.154717	67.833919	In Range	0.99895	4.0217	Pass

SAMPLE RESULT - Red Wine 41649

Peak Name	Retention Time (min)	Area (pA·s)	Conc. (ppm)	Calibration In Range	R^2	RSE (%)	Calibration Flag
acetaldehyde	4.2820776	7.4743238	79.954658	In Range	0.99939	3.46424	Pass
ethyl acetate	7.3934363	18.327038	58.857656	In Range	0.99864	4.98279	Pass
methanol	7.5759125	32.338699	186.01666	In Range	0.99916	3.65212	Pass
n-propanol	11.471873	10.467087	31.063011	In Range	0.99924	3.56421	Pass
amyl alcohols	16.921318	106.5695	278.42291	In Range	0.99999	2.59124	Pass
n-hexanol	21.597716	27.165207	70.485963	In Range	0.99895	4.0217	Pass

SAMPLE RESULT - Red Port Wine 41257

Peak Name	Retention Time (min)	Area (pA·s)	Conc. (ppm)	Calibration In Range	R^2	RSE (%)	Calibration Flag
acetaldehyde	4.2819952	9.7427208	104.49109	In Range	0.99939	3.46424	Pass
ethyl acetate	7.3920899	31.051252	100.0647	In Range	0.99864	4.98279	Pass
methanol	7.5773652	30.006694	172.12454	In Range	0.99916	3.65212	Pass
n-propanol	11.472759	18.645695	57.312401	In Range	0.99924	3.56421	Pass
amyl alcohols	16.918554	150.01848	392.67298	In Range	0.99999	2.59124	Pass
n-hexanol	21.598384	16.659639	42.913981	In Range	0.99895	4.0217	Pass

SAMPLE RESULT - Red Port Wine 41664 (Reserva Ruby)

Peak Name	Retention Time (min)	Area (pA·s)	Conc. (ppm)	Calibration In Range	R^2	RSE (%)	Calibration Flag
acetaldehyde	4.284452	3.0790561	32.412625	In Range	0.99939	3.46424	Pass
ethyl acetate	7.392726	13.619113	43.611157	In Range	0.99864	4.98279	Pass
methanol	7.5766822	37.764862	218.34118	In Range	0.99916	3.65212	Pass
n-propanol	11.471532	16.204161	49.476255	In Range	0.99924	3.56421	Pass
amyl alcohols	16.918756	123.23413	322.24294	In Range	0.99999	2.59124	Pass
n-hexanol	21.594649	6.2057134	15.477539	Low	0.99895	4.0217	Pass

SAMPLE RESULT - White Port Wine 41255

Peak Name	Retention Time (min)	Area (pA·s)	Conc. (ppm)	Calibration In Range	R^2	RSE (%)	Calibration Flag
acetaldehyde	4.2818248	7.4118473	79.278872	In Range	0.99939	3.46424	Pass
ethyl acetate	7.3922227	9.9195113	31.63009	In Range	0.99864	4.98279	Pass
methanol	7.576976	27.505174	157.22259	In Range	0.99916	3.65212	Pass
n-propanol	11.472128	19.003189	58.459784	In Range	0.99924	3.56421	Pass
amyl alcohols	16.919388	119.16824	311.55158	In Range	0.99999	2.59124	Pass
n-hexanol	21.597944	7.441695	18.721386	In Range	0.99895	4.0217	Pass

SAMPLE RESULT - Brandy 41831

Peak Name	Retention Time (min)	Area (pA·s)	Conc. (ppm)	Calibration In Range	R^2	RSE (%)	Calibration Flag
acetaldehyde	4.2826626	3.5722232	37.747036	In Range	0.99939	3.46424	Pass
ethyl acetate	7.392331	11.750508	37.559726	In Range	0.99864	4.98279	Pass
methanol	7.5807312	50.702861	295.41488	In Range	0.99916	3.65212	Pass
n-propanol	11.473089	34.398404	107.87101	In Range	0.99924	3.56421	Pass
amyl alcohols	16.923907	266.71211	699.52149	In Range	0.99999	2.59124	Pass
n-pentanol	17.946449	3.4680632	8.6568565	Low	0.99914	3.69317	Pass
n-hexanol	21.593071	6.6266864	16.582387	In Range	0.99895	4.0217	Pass

SAMPLE RESULT - Brandy 41832 (Brandy Constantino 36 Graus)

Peak Name	Retention Time (min)	Area (pA·s)	Conc. (ppm)	Calibration In Range	R^2	RSE (%)	Calibration Flag
acetaldehyde	4.2816138	3.2534052	34.298496	In Range	0.99939	3.46424	Pass
ethyl acetate	7.3925038	10.917616	34.862428	In Range	0.99864	4.98279	Pass
methanol	7.5793707	46.387872	269.70981	In Range	0.99916	3.65212	Pass
n-propanol	11.471575	31.265183	97.814875	In Range	0.99924	3.56421	Pass
amyl alcohols	16.923359	241.66061	633.64801	In Range	0.99999	2.59124	Pass
n-pentanol	17.946674	3.218254	7.986186	Low	0.99914	3.69317	Pass
n-hexanol	21.594136	6.0433455	15.051402	Low	0.99895	4.0217	Pass

SAMPLE RESULT - Brandy 41276 (Aguardente V-Verde Ferreirinha)

Peak Name	Retention Time (min)	Area (pA·s)	Conc. (ppm)	Calibration In Range	R^2	RSE (%)	Calibration Flag
acetaldehyde	4.2816869	21.79646	234.87206	In Range	0.99939	3.46424	Pass
ethyl acetate	7.3922183	105.7851	342.08839	In Range	0.99864	4.98279	Pass
methanol	7.5819583	24.521822	139.45029	In Range	0.99916	3.65212	Pass
n-propanol	11.47463	48.301993	152.49482	In Range	0.99924	3.56421	Pass
amyl alcohols	16.920529	363.54423	954.14374	In Range	0.99999	2.59124	Pass
n-pentanol	17.943122	1.5762395	3.5778192	Low	0.99914	3.69317	Pass
n-hexanol	21.563229	4.6080987	11.284581	Low	0.99895	4.0217	Pass

Results per analyte

The table below shows the sample analysis results presented for each component individually.

ANALYTE - Acetaldehyde

Peak Name	Retention Time (min)	Area (pA·s)	Conc. (ppm)	Sample Name
acetaldehyde	4.28295	7.4352275	79.531767	TVT_Red Wine 41648_001
acetaldehyde	4.2820776	7.4743238	79.954658	TVT_Red Wine 41649_001
acetaldehyde	4.2819952	9.7427208	104.49109	TVT_Red Port wine 41257_001
acetaldehyde	4.284452	3.0790561	32.412625	TVT_Red Port wine 41664_001
acetaldehyde	4.2818248	7.4118473	79.278872	TVT_White Port wine 41255_001
acetaldehyde	4.2826626	3.5722232	37.747036	TVT_Brandy 41831_001
acetaldehyde	4.2816138	3.2534052	34.298496	TVT_Brandy 41832_001
acetaldehyde	4.2816869	21.79646	234.87206	TVT_Brandy 41276_001

ANALYTE – Ethyl acetate

Peak Name	Retention Time (min)	Area (pA·s)	Conc. (ppm)	Sample Name
ethyl acetate	7.392872	17.715774	56.878091	TVT_Red Wine 41648_001
ethyl acetate	7.3934363	18.327038	58.857656	TVT_Red Wine 41649_001
ethyl acetate	7.3920899	31.051252	100.0647	TVT_Red Port wine 41257_001
ethyl acetate	7.392726	13.619113	43.611157	TVT_Red Port wine 41664_001
ethyl acetate	7.3922227	9.9195113	31.63009	TVT_White Port wine 41255_001
ethyl acetate	7.392331	11.750508	37.559726	TVT_Brandy 41831_001
ethyl acetate	7.3925038	10.917616	34.862428	TVT_Brandy 41832_001
ethyl acetate	7.3922183	105.7851	342.08839	TVT_Brandy 41276_001

ANALYTE – Methanol

Peak Name	Retention Time (min)	Area (pA·s)	Conc. (ppm)	Sample Name
methanol	7.5759954	30.979996	177.92265	TVT_Red Wine 41648_001
methanol	7.5759125	32.338699	186.01666	TVT_Red Wine 41649_001
methanol	7.5773652	30.006694	172.12454	TVT_Red Port wine 41257_001
methanol	7.5766822	37.764862	218.34118	TVT_Red Port wine 41664_001
methanol	7.576976	27.505174	157.22259	TVT_White Port wine 41255_001
methanol	7.5807312	50.702861	295.41488	TVT_Brandy 41831_001
methanol	7.5793707	46.387872	269.70981	TVT_Brandy 41832_001
methanol	7.5819583	24.521822	139.45029	TVT_Brandy 41276_001

ANALYTE – N-propanol

Peak Name	Retention Time (min)	Area (pA·s)	Conc. (ppm)	Sample Name
n-propanol	11.470724	9.7547181	28.776651	TVT_Red Wine 41648_001
n-propanol	11.471873	10.467087	31.063011	TVT_Red Wine 41649_001
n-propanol	11.472759	18.645695	57.312401	TVT_Red Port wine 41257_001
n-propanol	11.471532	16.204161	49.476255	TVT_Red Port wine 41664_001
n-propanol	11.472128	19.003189	58.459784	TVT_White Port wine 41255_001
n-propanol	11.473089	34.398404	107.87101	TVT_Brandy 41831_001
n-propanol	11.471575	31.265183	97.814875	TVT_Brandy 41832_001
n-propanol	11.47463	48.301993	152.49482	TVT_Brandy 41276_001

ANALYTE – Amyl alcohols (2-methyl-1-butanol + 3-methyl-1-butanol)

Peak Name	Retention Time (min)	Area (pA·s)	Conc. (ppm)	Sample Name
amyl alcohols	16.919089	102.11756	266.71643	TVT_Red Wine 41648_001
amyl alcohols	16.921318	106.5695	278.42291	TVT_Red Wine 41649_001
amyl alcohols	16.918554	150.01848	392.67298	TVT_Red Port wine 41257_001
amyl alcohols	16.918756	123.23413	322.24294	TVT_Red Port wine 41664_001
amyl alcohols	16.919388	119.16824	311.55158	TVT_White Port wine 41255_001
amyl alcohols	16.923907	266.71211	699.52149	TVT_Brandy 41831_001
amyl alcohols	16.923359	241.66061	633.64801	TVT_Brandy 41832_001
amyl alcohols	16.920529	363.54423	954.14374	TVT_Brandy 41276_001

ANALYTE – N-pentanol

Peak Name	Retention Time (min)	Area (pA·s)	Conc. (ppm)	Sample Name
n-pentanol	17.946449	3.4680632	8.6568565	TVT_Brandy 41831_001
n-pentanol	17.946674	3.218254	7.986186	TVT_Brandy 41832_001
n-pentanol	17.943122	1.5762395	3.5778192	TVT_Brandy 41276_001

ANALYTE – N-hexanol

Peak Name	Retention Time (min)	Area (pA·s)	Conc. (ppm)	Sample Name
n-hexanol	21.598748	26.154717	67.833919	TVT_Red Wine 41648_001
n-hexanol	21.597716	27.165207	70.485963	TVT_Red Wine 41649_001
n-hexanol	21.598384	16.659639	42.913981	TVT_Red Port wine 41257_001
n-hexanol	21.594649	6.2057134	15.477539	TVT_Red Port wine 41664_001
n-hexanol	21.597944	7.441695	18.721386	TVT_White Port wine 41255_001
n-hexanol	21.593071	6.6266864	16.582387	TVT_Brandy 41831_001
n-hexanol	21.594136	6.0433455	15.051402	TVT_Brandy 41832_001
n-hexanol	21.563229	4.6080987	11.284581	TVT_Brandy 41276_001

QC analysis results

Results for standard check sample

In addition to the samples, a standard check sample was added at the end of the sequence. The QC results for this standard check sample are presented in the table below.

CONTROL SAMPLE – Standard level 3

Peak Name	Retention Time (min)	Area (pA·s)	Conc. (ppm)	Std % Difference / % Drift	Std % Difference / % Drift Flag
acetaldehyde	4.282	3.573743	37.763476	-3.17	Pass
ethyl acetate	7.386	13.895116	44.504985	-1.1	Pass
methanol	7.576	7.4337399	37.653882	-4.91	Pass
n-propanol	11.47	13.394301	40.457957	1.14	Pass
amyl alcohols	16.912	31.709051	81.575684	-0.88	Pass
n-pentanol	18.311	15.27749	40.361988	-1.07	Pass
n-hexanol	21.556	15.527219	39.941933	-2.58	Pass

Summary

This report shows the analytical method settings, the calibration and the sample analysis result for the analysis of higher alcohols in wine and other alcoholic beverages by total vaporization technique and GC-FID. The analytes of interest are calibrated in the range of approximately 15 ppm to approximately 800 ppm with good linearity and good precision. Results for various samples are reported including wine, port wine and brandy. The calibration is checked by the analysis of a standard check sample at the end of the sample analysis sequence, the results of which are also presented in this report. For a level 3 standard, the difference from the expected concentration is found to be less than 5% or better for all analytes.

Analyses are done with:

HS 2400 and GC 2400